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Alcohol dehydrogenase 3 genotype modification of the association of alcohol consumption with breast cancer risk

Jo L. Freudenheim[1,*], Christine B. Ambrosone[2], Kirsten B. Moysich[3], John E. Vena[1], Saxon Graham[1], James R. Marshall[4], Paola Muti[1], Rosemary Laughlin[1], Takuma Nemoto[5], Lea C. Harty[6], G. Adam Crits[7], Arthur W.K. Chan[8] & Peter G. Shields[7,*]

[1]*Department of Social and Preventive Medicine, 270 Farber Hall, Buffalo, NY 14214, USA;* [2]*National Center for Toxicological Research, Division of Molecular Epidemiology, Jefferson, AR, USA;* [3]*Roswell Park Cancer Institute, Cancer Epidemiology and Prevention, Buffalo, NY, USA;* [4]*Arizona Cancer Center, Tucson, AZ, USA;* [5]*Department of Surgery, State University of New York, Buffalo, NY, USA;* [6]*Division of Cancer Epidemiology and Genetics;* [7]*Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD, USA;* [8]*Research Institute on Addictions, Buffalo, NY, USA (* Authors for correspondence)*

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Abstract

Objectives: Because alcohol dehydrogenase 3 (ADH_3) is rate-limiting in alcohol oxidation and is polymorphic, we examined ADH_3 genotype in relation to alcohol intake and breast cancer risk.
Methods: We conducted a case–control study among Caucasian women aged 40–85 with incident, pathologically confirmed breast cancer and controls, frequency-matched on age and county. Queries included alcohol intake in the past 20 years. Genomic DNA was genotyped for the exon VIII ADH polymorphism by PCR followed by restriction enzyme digestion. Computation of odds ratios (OR) and 95% confidence intervals (CI) was by unconditional logistic regression.
Results: We found increased risk among pre- (OR 2.3, 95%, CI 1.2–4.3) but not postmenopausal women (OR 1.1, 95% CI 0.7–1.7) associated with $ADH_3^{1\text{-}1}$ compared to $ADH_3^{1\text{-}2}$ and $ADH_3^{2\text{-}2}$ genotypes. Risk was increased for premenopausal women with the $ADH_3^{1\text{-}1}$ genotype and alcohol intake above the median (OR 3.6, 95% CI 1.5–8.8) compared to lighter drinkers with the $ADH_3^{2\text{-}2}$ or $ADH_3^{1\text{-}2}$ genotypes. ORs were close to null for premenopausal women in other drinking and genotype groups and for postmenopausal women categorized by genotype and alcohol consumption.
Conclusion: Among premenopausal women there may be a group more genetically susceptible to an alcohol consumption effect on breast cancer risk.

Introduction

While there is evidence that alcohol consumption may increase the risk of breast cancer [1–3], the mechanism of action is not well understood. It is possible that genetic differences in the metabolism of alcohol may alter the relation of alcohol exposure to breast cancer. Evaluation of heterogeneous groups may mask susceptible subgroups and impair estimation of effects. In this study we evaluated genetic variation in alcohol dehydrogenase, a key enzyme in alcohol metabolism, as a modifying factor in the relation between alcohol intake and breast cancer risk.

Alcohol dehydrogenase (ADH) catalyzes the oxidation of ethanol to acetaldehyde and plays a rate-limiting role in the metabolic pathway for most human ethanol oxidation. Dimeric class I ADH enzymes are composed of subunits encoded by genes designated as ADH_1, ADH_2, and ADH_3. Genetic variants with altered kinetic properties have been identified at the ADH_2 and ADH_3 loci [4]. The aldehyde dehydrogenase family of enzymes (ALDH) is also involved in alcohol metabolism, and

variant alleles with altered kinetic activities have been identified in the $ALDH_2$ gene [4]. Polymorphisms in ADH_2 and $ALDH_2$ are rare in Caucasian populations [4–6]. In one study of the ADH_3 gene, approximately 58%, 91% and 88% of European whites, Asians and Africans, respectively, had the $ADH_3{}^1$ allele [5]. For this study of Caucasians we examined effects of the ADH_3 polymorphism. There is evidence that this variant has functional importance. *In vitro* there is more than two-fold difference in V_{max} between the ADH_3 genotypes [4], with the $ADH_3{}^1$ allele coding for the more rapid form of the enzyme.

While there are, to our knowledge, no reports on the association of ADH_3 in relation to breast cancer, there have been reports of an association of the $ADH_3{}^{1\text{-}1}$ genotype with increased risk of cancer of the oral cavity and pharynx [7, 8] and of hepatic cirrhosis and chronic pancreatitis [6]. We report here on the results of a case–control study of breast cancer risk with an examination of associations of alcohol consumption stratified by ADH_3 genotype.

Materials and methods

We conducted a case–control study of breast cancer in pre- and postmenopausal women in western New York State. All participants provided written informed consent; procedures for protection of human subjects in this study were approved by the Human Subjects Review Board of the State University of New York at Buffalo School of Medicine and Biomedical Sciences and of each of the participating hospitals. The women in the study were between the ages of 40 and 85, residents of Erie and Niagara counties, alert, able to speak English and in sufficiently good health to be interviewed; all were Caucasian. Women were considered to be premenopausal if they were currently menstruating, if they were not menstruating because of a hysterectomy or other medical intervention, or if they had at least one of their ovaries and were less than age 50. All other women were considered to be postmenopausal.

Women with incident, primary, histologically confirmed breast cancer were identified from pathology records of all the major hospitals in the two counties; case ascertainment was conducted in the period beginning November 1986 and ending October 1989 for postmenopausal cases, and ending April 1991 for premenopausal cases. The physician of each woman identified with breast cancer was contacted to obtain consent to allow us to invite the woman for an interview. Of eligible cases, 66% of premenopausal and 54% of postmenopausal cases were interviewed. Physician refusal to allow us to contact their patients accounted for most of the lack of participation, 74% and 71% of non-participation for pre- and postmenopausal women, respectively. Interviews were conducted, on average, 2 months after diagnosis; no interviews were conducted more than 1 year after diagnosis.

Controls were frequency-matched to cases on age and county. The listing of licensed New York State drivers was used for random selection of women under age 65; women age 65 and over were randomly selected from the listing of the Health Care Finance Administration. Sixty-two percent of the eligible premenopausal and 44% of eligible postmenopausal controls were interviewed. Because controls under age 65 were licensed drivers, we asked the cases under 65 if they had driver's licenses. Nine did not hold a driver's license. Compared to cases with licenses, women without licenses were slightly less educated and slightly, though not significantly, older. All are included in these analyses. For a subset of participating controls and those refusing to participate, we conducted a very brief phone interview querying usual frequency of consumption of several foods. These participants and non-participants did not differ in reported intake of vegetables, fruits, meat or coffee. Non-participants were somewhat more likely to smoke. Information was not collected on alcohol intake in this comparison of participants and non-participants [9, 10].

Interviews

Interviews were conducted in the participants' homes by trained interviewers. The interview lasted, on average, 2 hours. Details of the interview have been described elsewhere [9–11]. Included in the interview were questions regarding usual diet 2 years before the interview, reproductive history, medical history, family history of cancer, smoking history (pack-years) and other breast cancer risk factors. Body mass index (BMI) was calculated from reported height and weight, as weight (kg)/height^2 (m^2). Family history of breast cancer was defined as having at least one first-degree relative (mother, sister, daughter) with breast cancer.

Questions regarding alcohol intake included queries of the usual frequency of intake and number of drinks per occasion for wine, beer and hard liquor during the year 2 years ago, 10 years ago, 20 years ago and at age 16. Total alcohol intake was calculated as the sum of the reported number of drinks of beer, wine and hard liquor under the assumption that the alcohol content of one glass of beer or wine or one shot of hard liquor was approximately the same. An index of usual alcohol consumption in the last 20 years was estimated as a

weighted sum of the reported intakes for 2 years ago, 10 years ago and 20 years ago.

At the end of the interview, participants were asked to provide a blood sample following an additional informed consent. About 45% of premenopausal and 63% of postmenopausal participants agreed to give a blood sample.

Molecular genetic analyses

All analyses were conducted at the Laboratory for Human Carcinogenesis at the National Cancer Institute. DNA was extracted from blood clots [11]. As previously described [7], a 145 bp fragment including the Exon VIII polymorphism was amplified by the polymerase chain reaction (PCR) using a modification of the method of Groppi *et al*. [12]. The highly homologous ADH_1 and ADH_2 genes were digested with the NlaIII restriction enzyme prior to the PCR. An aliquot of this digestion mixture was then subjected to PCR and subsequent SspI enzymatic digestion to reveal the ADH_3 genotype (*i.e*., $ADH_3{}^{1\text{-}1}$, $ADH_3{}^{1\text{-}2}$, or $ADH_3{}^{2\text{-}2}$). Every 14 samples contained a positive and negative control. The results were scored separately by two authors, independently, who were blinded to all identifying data including subjects' case–control status. Twenty percent of samples were repeated for quality control. In the adjusted analyses, NAT2 genotype was examined as a potential adjusting variable; methodology for the NAT2 analyses has been described previously [11].

The final sample for this report included 134 premenopausal cases and 126 premenopausal controls, 181 postmenopausal cases and 230 postmenopausal controls, those women whom we interviewed and whose ADH_3 genotype could be determined. Because we did not obtain blood samples from all participants who completed the interview, nor were we able to successfully determine the ADH_3 polymorphism on all blood samples, we compared the characteristics of those included in this report with the entire group included in the case–control study; comparisons of means were made using Student's *t*-test. Those with and without ADH_3 data were largely similar, with a few exceptions. Differences ($p < 0.05$) among premenopausal women were that those with data tended to be older, have higher parity and to drink less beer than those without. Among postmenopausal women, the only characteristic that was significantly different was age; those with ADH_3 data were older.

Statistical analysis

Because there are indications that there are differences in the risk factors for pre- and postmenopausal breast cancer [13], and in particular because there may be differences in the effect of alcohol intake depending on menopausal status [1], analyses were stratified by menopausal status. For potential confounding factors, means and standard deviations for groups defined by ADH_3 genotype and by case–control status were compared by one-way analysis of variance, with a two-tailed test of significance; values for categorical data were compared using the chi-square test [14]. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using unconditional logistic regression [15]. For analyses of categorical data, ORs were calculated relative to the indicated referent category. Cutoffs for categories of alcohol intake were at the median level of intake for controls. Because of limitations in sample size it was not possible to examine groups with more narrowly defined alcohol intakes. Adjusted analyses included control for age, education, family history of breast cancer, reported history of benign breast disease, body mass index (BMI), parity, age at first birth, age at menarche, fruit and vegetable intake, duration of lactation and (for postmenopausal women) age at menopause. Most of these factors were examined for confounding effects because they have been found to be associated with risk of breast cancer. We also examined possible confounding by smoking history, NAT2 status and smoking by NAT2 interaction because we had previously found these to associated with risk in this population [11]. ORs for the ADH_3 genotypes were calculated and then ORs for alcohol intake both without and with stratification on ADH_3 genotype were calculated. Because of issues regarding differential recall for cases and controls in case–control studies, we also examined a case–case analysis in relation to alcohol dehydrogenase status; alcohol intake was regressed on ADH genotype among the cases with the $ADH_3{}^{2\text{-}2}$ and $ADH_3{}^{1\text{-}2}$ groups combined as the referent with comparison to $ADH_3{}^{1\text{-}1}$ [16, 17].

Results

For all analyses the cutoff between the lower and higher groups of drinkers was at the median for controls, 6.5 and 4.4 drinks per month on average over the past 20 years, for the pre- and postmenopausal women, respectively. The associations between reported alcohol consumption in the past 20 years and risk of breast cancer in this sample of individuals with available genetic data are shown in Table 1. For both pre- and postmenopausal women, confidence intervals included the null. For the premenopausal women there was a suggestion of increased risk among heavier drinkers. Similar results

Table 1. Alcohol consumption in the past 20 years and risk of breast cancer, Western New York, 1987–91 (subgroup of women with alcohol dehydrogenase 3 (ADH_3) genotype measured)

Alcohol*	Cases	Controls	Crude OR	Adjusted OR†	95% CI†
Premenopausal					
Lower	54	63	1.0	1.0	
Higher	80	63	1.5	1.6	(0.9–2.6)
Total	134	126			
Postmenopausal					
Lower	93	113	1.0	1.0	
Higher	88	117	0.9	0.9	(0.6–1.5)
Total	181	230			

* Cutoffs for lower and higher groups of drinkers were the medians of the reported average consumption over the past 20 years; the cutoff was 6.5 and 4.4 drinks per month, for pre- and postmenopausal women, respectively.

† OR = odds ratio; CI = confidence interval. Adjusted for age, education, family history of breast cancer, history of benign breast disease, BMI, parity, age at first birth, age at menarche, fruit and vegetable intake, alcohol intake in the past 20 years, lifetime duration of lactation, and age at menopause (postmenopausal women only).

were obtained when all the data, including participants who did not provide a blood sample, were analyzed. We also examined risk associated with alcohol consumption separately for the reports of alcohol consumption 2 years ago, 10 years ago and 20 years ago. ORs for these periods were similar to those shown for the combined index; confidence intervals overlapped for all three periods for pre- and postmenopausal women.

In Table 2, breast cancer risk factors are shown for cases and controls grouped by genotype. In general, characteristics of the three genotype groups within the cases and the controls were similar. For alcohol intake, values shown are for all subjects combined, including non-drinkers. For the premenopausal women, 4% of cases and 6% of controls were non-drinkers; for postmenopausal women, non-drinkers constituted 13% of cases and 11% of controls. The percentage of non-drinkers did not differ by genotype in any of the groups defined by case–control and menopausal status. In one-way analysis of variance the reported alcohol intakes were not different by genotype for either the pre- or postmenopausal controls. For premenopausal cases with the $ADH_3^{2\text{-}2}$ genotype, reported alcohol intakes were significantly higher than those with the $ADH_3^{1\text{-}2}$ genotype ($p < 0.05$), but not the $ADH_3^{1\text{-}1}$ genotype. There were also some differences in smoking history between the homozygotes and the heterozygotes among the premenopausal women. Among postmenopausal women, alcohol consumption and smoking did not differ for the different groups; there was a difference by genotype for education among the cases.

In Table 3, risk of breast cancer associated with ADH_3 genotype is shown. There was an increase in risk for the premenopausal women associated with the $ADH_3^{1\text{-}1}$ genotype; the confidence interval included the null value (adjusted OR 2.0, 95% CI 0.8–4.6). There was little evidence of an association of genotype with risk for postmenopausal women. ORs estimated without adjusting for alcohol intake were similar to those shown here. Addition of smoking, NAT2 and an interaction term of NAT2 and smoking did not appreciably change the estimates.

We also examined risk of breast cancer associated with the $ADH_3^{1\text{-}1}$ genotype when the referent was the $ADH_3^{2\text{-}2}$ and $ADH_3^{1\text{-}2}$ genotype groups combined. For premenopausal women the OR was 2.3 (95% CI 1.2–4.3); for postmenopausal women the OR was 1.1 (95% CI 0.7–1.7) (data not shown).

In Table 4, ORs for alcohol intake by ADH_3 genotype are shown. The referent was women with lower intake of alcohol and either the $ADH_3^{2\text{-}2}$ or $ADH_3^{1\text{-}2}$ genotype. (We also analyzed these data with $ADH_3^{2\text{-}2}$ alone as the referent. The results were similar to those shown here. However, the findings were less stable because the sample size in the reference group was small and CI were wider.) Among the premenopausal women, ORs were generally close to the null and CIs included the null for all categories with one exception. Among women who drank more than the median intake and who had the $ADH_3^{1\text{-}1}$ genotype, the OR was 3.6 with 95% CI 1.5–8.8. It appeared that the effect associated with both the $ADH_3^{1\text{-}1}$ genotype and higher alcohol consumption was more than additive; however, the multiplicative interaction term in a logistic regression was not significantly different from the null ($p = 0.16$). The estimates of risk in Table 4 were essentially unchanged when smoking, NAT2 and smoking × NAT2 were included in the model. We also examined risk associated with alcohol within the group of women with the $ADH_3^{1\text{-}1}$ genotype. With lighter drinkers as the referent, the adjusted OR for drinking more than the median of alcohol was 3.9, 95% CI 1.3–10.1 (data not shown). Additionally, we repeated this latter analysis, changing the cutpoint for the low and high drinkers so that there was an even distribution within the premenopausal controls with the $ADH_3^{1\text{-}1}$ genotype. The results were essentially the same (OR 3.9, 95% CI 1.4–10.9).

Among postmenopausal women there was no evidence of an association of alcohol intake and risk when modification by ADH_3 was taken into account. Because of reports that an increased risk associated with alcohol consumption among postmenopausal women may be restricted to those who have used estrogen replacement therapy (ERT) [18, 19], we also looked at the OR among

Table 2. Characteristics of study sample by case and control status and alcohol dehydrogenase 3 (ADH_3) genotype

Characteristic*	Cases			Controls		
	ADH_3^{1-1}	ADH_3^{1-2}	ADH_3^{2-2}	ADH_3^{1-1}	ADH_3^{1-2}	ADH_3^{2-2}
Premenopausal women						
Age (years)	46.2 (4.6)	46.8 (4.0)	45.0 (3.2)	46.2 (3.2)	46.9 (3.7)	47.5 (4.5)
Education (years)	13.6 (2.5)	14.0 (3.2)	14.1 (2.5)	14.1 (2.4)	13.6 (2.8)	14.2 (2.6)
Age at menarche (years)	12.6 (1.8)	12.4 (1.6)	12.5 (1.2)	13.1 (1.9)	12.9 (1.7)	13.3 (1.7)
Body mass index†	24.2 (5.2)	25.2 (6.0)	24.7 (4.8)	25.2 (4.6)	25.6 (4.1)	26.6 (6.0)
History of benign breast disease (percentage of cases or controls)	22	20	7	15	13	9
Family history of breast cancer (percentage of cases or controls)	8[a]	8[b]	2[a,b]	0.8	0.4	0.8
Total alcohol† (drinks/month)	16.8 (20.2)	9.7[c] (11.3)	20.2[c] (24.4)	14.2 (28.0)	13.4 (18.1)	12.5 (12.8)
Duration lactation (months)	4.0 (8.6)	2.1 (4.3)	2.7 (5.0)	8.0 (14.1)	5.2 (12.1)	6.2 (13.1)
Parity	2.5 (1.6)	2.1 (1.5)	1.9 (1.3)	2.4 (1.6)	2.8 (1.8)	3.0 (1.7)
Age at first birth (years)	24.0 (4.4)	24.0 (4.8)	23.8 (5.2)	22.8 (4.0)	21.9 (4.1)	21.8 (4.0)
Vegetable intake† (g/day)	459 (220)	395 (180)	419 (175)	462 (190)	473 (201)	450 (155)
Fruit intake† (g/day)	239 (133)	210 (141)	170 (125)	272 (170)	245 (149)	216 (112)
Smoking (pack-years)	11.7[d] (16.4)	5.8[d] (10.1)	12.6 (14.4)	5.7[e] (11.4)	11.7[e,f] (16.6)	4.8[f] (8.9)
n	63	50	21	42	60	24
Postmenopausal women						
Age (years)	64.9 (6.4)	63.6 (7.8)	61.9 (7.5)	63.4 (7.7)	63.1 (7.2)	61.6 (6.7)
Education (years)	12.2[e] (2.6)	12.3 (2.9)	13.4[e] (3.2)	12.3 (2.6)	12.0 (2.3)	12.7 (2.5)
Age at menarche (years)	13.0 (1.8)	13.0 (1.6)	12.6 (1.4)	12.7 (1.7)	13.1 (1.6)	12.6 (1.3)
Age at menopause (years)	47.8 (5.3)	47.6 (6.1)	46.8 (5.5)	46.2 (6.0)	47.6 (5.3)	47.0 (6.0)
Body mass index†	25.7 (5.3)	26.0 (5.0)	25.6 (3.6)	25.2 (4.2)	25.7 (5.4)	25.4 (4.7)
History of benign breast disease (percentage of cases or controls)	6	12	2	8	8	3
Family history of breast cancer (percentage of cases or controls)	6	6	5	3	6	1
Total alcohol† (drinks/month)	11.8 (21.9)	17.1 (31.4)	17.7 (29.8)	10.6 (16.6)	15.9 (25.2)	12.6 (15.2)
Duration of lactation (months)	3.5 (5.7)	4.6 (11.0)	4.0 (8.0)	6.2 (10.7)	4.2 (9.0)	5.1 (10.0)
ERT (percentage ever used of cases or controls)	9	11	6	10	15	9
Parity	3.1 (2.0)	2.7 (2.0)	3.2 (2.8)	2.8 (2.2)	3.1 (2.0)	2.9 (1.8)
Age at first birth (years)	24.8 (5.0)	24.1 (4.9)	23.4 (5.2)	23.3 (4.6)	23.5 (4.6)	23.3 (3.8)
Vegetable intake† (g/day)	451 (201)	406 (175)	417 (207)	458 (237)	456 (227)	484 (334)
Fruit intake† (g/day)	298 (175)	254 (175)	287 (177)	306 (186)	282 (172)	308 (218)
Smoking (pack-years)	14.6 (21.0)	17.4 (21.3)	16.9 (29.1)	12.9 (16.5)	13.8 (19.3)	13.0 (23.0)
n	64	89	28	81	114	35

* Values shown are mean (SD) except for history of benign breast disease and family history of breast cancer which are percentages with positive history. Two-sided comparisons of means between the ADH_3 groups within cases or controls were computed by ANOVA; comparisons of categories were with the chi-square test. Those with the same letter are significantly different, $p < 0.05$.

† Body mass index (kg/m^2) calculated from reported height and weight 2 years before the interview. Alcohol values are average drinks per month during the past 20 years, calculated from the weighted sum of reported consumption 2, 10 and 20 years ago; values include non-drinkers. Vegetable and fruit intake is reported intake in the year 2 years before the interview.

women who had ever used ERT. Among the heavier drinkers with the ADH_3^{1-1} genotype compared to lighter drinkers with the other ADH_3 genotypes for women who had ever used ERT the adjusted OR was 1.2 and the 95% CI 0.8–1.7; for those who had never used ERT the OR was 1.0 and the 95% CI 0.9–1.6. Sample size was quite small for the cells in these analyses; there were only 10 cases and 9 controls with the ADH_3^{1-1} genotype that had ever used ERT. All of these analyses were based on reports of alcohol consumption in the past 20 years. We had also queried regarding alcohol intake at age 16. The number of drinkers at that age was too small to estimate whether there was a modifying effect of ADH_3 genotype.

In a case–case analysis we examined risk associated with the ADH_3^{1-1} genotype compared to the combined ADH_3^{1-2} and ADH_3^{2-2} groups. As for the case–control analyses, there was evidence of some increase in risk

Table 3. Alcohol dehydrogenase 3 polymorphisms and risk of breast cancer, Western New York, 1987–91

ADH_3	Cases	Controls	Crude OR	Adjusted OR*	95% CI*
Premenopausal					
2-2	21	24	1.0	1.0	
1-2	50	60	1.0	0.8	(0.4–1.8)
1-1	63	42	1.7	2.0	(0.8–4.6)
Total	134	126			
Postmenopausal					
2-2	28	35	1.0	1.0	
1-2	89	114	1.0	1.1	(0.6–2.1)
1-1	64	81	1.0	1.2	(0.6–2.3)
Total	181	230			

* OR = odds ratio; CI = confidence interval. Adjusted for age, education, alcohol intake, family history of breast cancer, history of benign breast disease, BMI, parity, age at first birth, age at menarche, fruit and vegetable intake, lifetime duration of lactation and age at menopause (postmenopausal women only).

Table 4. Lifetime alcohol consumption by ADH_3 genotype and risk of breast cancer, Western New York, 1987–91

Alcohol*	Cases	Controls	Crude OR	Adjusted OR†	95% CI†
Premenopausal					
$ADH_3^{2\text{-}2}$ + $ADH_3^{1\text{-}2}$					
Lower	33	38	1.0	1.0	
Higher	38	46	1.0	0.8	(0.4–1.7)
$ADH_3^{1\text{-}1}$					
Lower	21	25	1.0	1.0	(0.4–2.5)
Higher	42	17	2.8	3.6	(1.5–8.8)
Postmenopausal					
$ADH_3^{2\text{-}2}$ + $ADH_3^{1\text{-}2}$					
Lower	60	69	1.0	1.0	
Higher	57	80	0.8	0.8	(0.5–1.4)
$ADH_3^{1\text{-}1}$					
Lower	34	46	0.8	0.9	(0.5–1.6)
Higher	30	35	1.0	1.2	(1.1–2.2)

* Cutoffs for lower and higher groups of drinkers were the medians of the reported average consumption over the past 20 years; the cutoff was 6.5 and 4.4 drinks per month, for pre- and postmenopausal women, respectively.

† OR = odds ratio; CI = confidence interval. Adjusted for age, education, family history of breast cancer, history of benign breast disease, BMI, parity, age at first birth, age at menarche, fruit and vegetable intake, duration of lactation and age at menopause (postmenopausal women only).

associated with the $ADH_3^{1\text{-}1}$ genotype for pre- but not postmenopausal women. For premenopausal women, risk was more than doubled for women drinking more than the median compared to lighter drinkers (adjusted OR 2.5, 95% CI 1.1–8.4). For the postmenopausal women the adjusted OR was 1.0 and the 95% CI was 0.4–2.1 (data not shown).

Discussion

This study of women in western New York provides evidence that the association of alcohol consumption with breast cancer risk may differ depending on genotype. Among premenopausal women, we found an increase in risk of more than 3.5-fold for drinkers above the median with the $ADH_3^{1\text{-}1}$ genotype. We did not find an increase in risk for heavier drinkers with the other genotypes. Further, we did not find any indication in this population of generally light drinkers of a modifying effect of ADH_3 genotype among postmenopausal women. To our knowledge this is the first study of the relation of the ADH_3 polymorphism with alcohol and breast cancer risk. As noted above, there is some indication of an increase in risk of other alcohol-related diseases among individuals with the $ADH_3^{1\text{-}1}$ genotype, including reports of a 2.5–6-fold increase in risk of oral and pharyngeal cancer [7, 8]. There are a considerable number of studies that indicate that alcohol is related to increased risk of breast cancer [1–3]. Some [20–24], but not all [1] studies find risk associated with alcohol intake particularly among premenopausal women.

This modification of the association between alcohol consumption and risk of breast cancer by ADH_3 genotype may provide some indication as to the mechanism of effect of alcohol exposure. Alcohol metabolism in humans is regulated primarily by the ADH system of enzymes. There is considerable evidence that acetaldehyde, the product of alcohol dehydrogenase oxidation of alcohol, has carcinogenic properties [25]. Acetaldehyde is mutagenic and carcinogenic in experimental animals. In short-term cell culture assays, including assays of human cells, acetaldehyde but not ethanol is mutagenic [26, 27]. *In vitro*, acetaldehyde effects include DNA adducts [28, 29], DNA crosslinks and DNA–protein crosslinks [30, 31] and inhibition of DNA repair [30]. The International Agency for Research on Cancer (IARC) has indicated that the evidence regarding acetaldehyde is sufficient for it to be designated as a carcinogen in experimental animals [32]. *In vitro* the V_{max} for $ADH_3^{1\text{-}1}$ is more than 2-fold greater than for $ADH_3^{2\text{-}2}$ [4], and may therefore contribute to increased exposure to acetaldehyde. It should be noted, however, that in one study in Caucasians no difference was found in blood ethanol levels for different ADH_3 genotypes [33]. There is evidence of measurable levels of circulating acetaldehyde in pre-

menopausal women after consumption of moderate amounts of alcohol during the high estrogen phases of the menstrual cycle [34, 35]. There is also evidence of acetaldehyde excretion in human milk [36]; however, the determinations in milk were not made in conjunction with alcohol consumption. ADH_3 expression is greatest in the liver; however, there is evidence of ADH_3 activity in other organs [37–42] with an indication of expression, particularly in epithelial cells [41].

Another possible mechanism involving ADH and alcohol is with regard to steroid hormone metabolism. There is strong evidence that estrogen exposure is an important contributor to breast cancer risk [43]. Alcohol consumption appears to affect estrogen levels; there is evidence that both acute [44–46] and chronic [47–49] alcohol consumption lead to increased estrogen levels in premenopausal women and in postmenopausal women who take exogenous estrogen. ADH_3 is also involved in steroid hormone metabolism and is inhibited by testosterone [50, 51]. If the association of ADH_3 with risk is the result of an interaction with steroid hormones, that mechanism might explain why we saw an association with risk only among the premenopausal women. Given the toxic effects of acetaldehyde, the apparent likelihood of exposure to breast tissue of acetaldehyde and the interactions of alcohol, ADH_3 and estrogens, these mechanisms together may explain, at least in part, an association of alcohol consumption with breast cancer risk. Of course, there are other possible mechanisms that may also explain the association of alcohol with breast cancer risk, which also need to be considered.

In interpretation of these findings, several potential sources of error need to be considered. In this study, all measures of alcohol intake were by self-report, and measurement error is of concern. However, there is some evidence that reliability of recall of intake of alcohol in the past 5–10 years is relatively good [52, 53], although current drinking practices may bias recall of intake [52]. In data such as ours, there is also the concern of recall bias, that women with recently diagnosed breast cancer may report their previous alcohol intake differently than the healthy controls do. In one study, this potential source of bias accounted for only a small reduction in the relative risk estimate with bias toward the null [54]. As for the measure of ADH_3 status, there may also be some misclassification of the clinically significant ethanol oxidation phenotype. Methodologically, however, laboratory personnel were blinded to case–control status; error with regard to ADH_3 status would be non-differential and would contribute to an attenuation of the odds ratio estimate [55].

In terms of the selection of the sample, while every effort was made to include a population-based sample in this study, there were several sources of non-participation. For the cases, the largest source of non-participation was the refusal of physicians to allow us to contact the women. It may be that this lack of inclusion reflects physician rather than patient characteristics, but we could not verify whether or not this was true. Among the controls we do have some evidence that, at least for dietary intake, there were no differences among participants and those who did not participate [9, 10]. There may have been differences in alcohol intake of those refusing to participate; in particular it is possible that the heaviest drinkers in the population were underrepresented. For both cases and controls there is no reason to believe that participation would be related to ADH_3 polymorphism; the frequency of the $ADH_3{}^1$ and $ADH_3{}^2$ alleles measured in this population (59% and 41%, respectively, among the controls) were similar to those reported by others [4, 5, 56]. ADH_3 would be unlikely to affect alcohol consumption; studies of ADH_3 in Caucasians have not shown there to be differences in risk of alcoholism associated with the ADH_3 genotype [6, 56]. We did not find any difference in alcohol intake by ADH_3 polymorphism among the controls. Among premenopausal cases reported alcohol intake was lower for the $ADH_3{}^{1\text{-}2}$ genotype than for the $ADH_3{}^{2\text{-}2}$; this finding does not make biological sense in the context of the other groups where there were no differences. It may be that this apparent difference is the result of chance. There were no differences in intake for the other comparisons within the cases, for the controls or for the postmenopausal cases or controls.

Possible confounding is also of concern. Known risk factors for breast cancer were examined as potential confounders. The possibility remains that there were other correlated exposures that may explain the observed associations. In particular, it could be that there is confounding by genetic admixture even within this group of Caucasians. In addition, rather than the observed associations being an effect of ADH_3, it could be that the observed association is the result of linkage disequilibrium of ADH_3 with another gene causally related to breast cancer.

Finally, there is also the possibility that these findings were the result of chance. Given the small samples in some of the cells of analysis, and given the issues of potential bias, these results necessarily need to be considered as preliminary and await confirmation by other, larger epidemiologic studies. Because of the restriction by sample size we were only able to categorize participants into two levels of drinking. The group of heavier drinkers necessarily included women whose

alcohol consumption was in fact rather low. Additionally, the group of lighter drinkers included both non-drinkers and those who drink less frequently. With larger sample size and the ability to examine risk in groups that are more narrowly defined by alcohol consumption, it would have been possible to elucidate the association of drinking, genotype and risk.

Our data suggest that genetic differences in alcohol metabolism by ADH_3 should be considered as possible modifiers of the association between alcohol intake and breast cancer. In other studies, consideration of genetic variation in ADH_2 and ALDH, which we were not able to study, is warranted. Further, given the small number of individuals in some of the genotype–alcohol categories, chance may explain the findings; replication in other populations would be of importance. Our findings of an apparent modification of effect by ADH_3 genotype, if confirmed in other studies, would shed some light on the possible mechanism of an alcohol consumption effect on breast cancer and indicate a high-risk group for an alcohol effect.

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